PAOLO MANITTO and GIOVANNA SPERANZA (*)

Phytochemical Studies on Aloe (***)

Bitter aloe is the solid residue obtained by evaporating the liquid which drains from the leaves of certain plants of the genus Aloe (Liliaceae).

This genus is native to Africa, from the Cape to about 15° N to the southern parts of the Arabian Peninsula, and comprises over 300 species, to which additions are continually being made. Bitter aloe has been used since ancient times because of its purgative properties, and at present the exudates of a few species are commercially available as both drugs (Aloe paste, Acibar) and cosmetics (Aloe Gel).

The term “Cape aloe” refers to the product obtained from A. ferox Miller and its hybrids with A. africana Miller and A. spicata Baker, whereas the drug named “Barbados aloe” (or “Curacao aloe”) is derived from A. barbadensis Miller (= A. vera Tourn. ex Linn.). This species has spread in cultivation throughout the Mediterranean and from the West Indies and American mainland to India and Japan in the east (Fig. 1).

Among other species used locally in popular medicine we can mention A. arborescens Miller and A. saponaria Haw., which have been widely investigated by Japanese researchers. From a phytochemical point of view, extracts and exudates of a large number of Aloe sp. (ca. 240) have been qualitatively examined by Reynolds, using the TLC technique. This author also discussed the distribution of known compounds [1].

This overview deals with the structure and the biogenesis of secondary metabolites occurring in commercial samples of dried exudates as well as in whole plants. Particular emphasis will be given to the isolation and chemical characterization of new compounds performed by our research group (Chemistry

(*) Dipartimento di Chimica Organica e Industriale dell'Università di Milano, Milan, Italy.
(***) Presented at the International Congress on « Medicinal Plants » (Sansepolcro (AR), 17-19 October - Roma, October 20th 1987), organized by the Accademia Nazionale delle Scienze detta dei XL.
A. ferox Miller
A. spicata Baker  →  Cape aloe, Kenya aloe (paste)
A. africana Miller  →  purgative drug
                 →  bittering agent
A. barbadensis Miller  →  Barbados aloe, Curaçao aloe (paste)
(= A. vera Linn.)  →  purgative drug
                 →  bittering agent
Aloe gel
                 →  cosmetic use

A. saponaria Haw  →  « Shabonrokai »
                 →  popular medicine (Japan)

A. arborescens Miller  →  « Kidachirokai »
var. natalensis  →  popular medicine (Japan)

Fig. 1 - Principal Aloe spp. used for their medicinal, flavouring and cosmetic properties.

Department of the University and Centro Studi M. Branca, Milan, Italy) [2-6]. Some of these compounds are now under investigation for their organoleptic and potential therapeutic properties.

The main secondary metabolites so far found in aloe drugs as well as in fresh plants belong to four structural (and biogenetic) families:
1) 5-methylchromones; 2) 6-phenyl-2-pyrones; 3) anthracene derivatives; 4) naphtalene derivatives.

The most representative compound of each family is shown in Fig. 2.

5-methylchromones

A number of products having a chromone nucleus have been isolated from aloe drug or fresh plants (Fig. 3). All of them are characterized by the presence of a methyl group in 5-position and a three-carbon side-chain in 2-position. In addition, with the exception of the poorly distributed aloecone [7], all contain a β-D-glucopyranosyl residue linked to C-8 by a C-C bond.

Aloesin (also known as aloeresin B) was first isolated in pure form in 1970 by Haynes from Cape aloe and its structure elucidated on the basis of proton magnetic resonance data [8]. However, the position of the sugar moiety and the configuration of the anomeric carbon atom (C-1') remained undetermined.

Such structural details were unequivocally clarified by us as shown in Fig. 4, using more sophisticated techniques, i.e., 13-C nuclear magnetic resonance, spin decoupling and nuclear Overhauser effect. Furthermore, these spectroscopic
Fig. 2: Representative metabolites of Alor vpl.

ALOGEN A

ISOLETHOREL O-GLUCOSIDE

ALOESIN

ALOIN A AND B
<table>
<thead>
<tr>
<th>$R^1$</th>
<th>$R^2$</th>
<th>$R^3$</th>
<th>Name</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>H</td>
<td>CH$_2$COME</td>
<td>ALOESONE</td>
<td>(HOLDSTHOR, 1972)</td>
</tr>
<tr>
<td>H</td>
<td>Z</td>
<td>CH$_2$COME</td>
<td>ALOESIN (ALOERESIN B)</td>
<td>(HAYNES, 1970)</td>
</tr>
<tr>
<td>H</td>
<td>Y</td>
<td>CH$_2$COME</td>
<td>ALOERESIN A</td>
<td>(MANITO, 1982)</td>
</tr>
<tr>
<td>H</td>
<td>X</td>
<td>CH$_2$COME</td>
<td>FERULOYL-ALOERESIN</td>
<td>(MAKINO, 1974)</td>
</tr>
<tr>
<td>X</td>
<td>Y</td>
<td>CH$_2$COME</td>
<td>ALOERESIN C</td>
<td>(SPERANZA, 1985)</td>
</tr>
<tr>
<td>Me</td>
<td>Y</td>
<td>CH$_2$CHOH Me</td>
<td>ALOERESIN D</td>
<td>(SPERANZA, 1986)</td>
</tr>
<tr>
<td>H</td>
<td>W</td>
<td>CH$_2$COME</td>
<td>ISO-ALOERESIN A</td>
<td>(SPERANZA, 1987)</td>
</tr>
<tr>
<td>H</td>
<td>V</td>
<td>CH$_2$COME</td>
<td>METHOXYCINNAMOYL-ALOESIN</td>
<td>(MEBE, 1987)</td>
</tr>
</tbody>
</table>

Fig. 3 - 5-Methylchromones.
methods allowed us to demonstrate that aloesin A is the 2\'-O-(E)-p-coumaryl derivative of aloesin [2], thus rejecting the structure, previously suggested by Wagner in 1970, in which the acyl group was located in 6\'-O-position [9].

One of the most difficult problems encountered in the structural elucidation of compounds of the aloesin series [3, 4, 6, 10, 11] was the determination of the absolute configuration of the asymmetric C-10 in aloesin D. The R-configuration was proved only via chemical degradation as shown in Fig. 5. The methyl 3-hydroxybutanoate arising, in very low yield, from a drastic alkaline degradation of aloesin D, followed by diazomethane esterification, was converted
Fig. 5 - Chemical degradation of aloeresin D.
into the corresponding N-(1-phenylethyl)carbamate and N-(1-(1-naphthyl)ethyl)carbamate by reaction with optically pure R-(+)-1-phenylethylisocyanate and R-(−)-1-(1-naphthyl)ethylisocyanate, respectively. The carbamates were then shown to be diastereomically pure by gas-chromatographic and liquid-chromatographic comparison with samples prepared from racemic methyl 2-hydroxybutanoate; in addition, their peak resulted coincident with that of the carbamates obtained from an authentic sample of (R)-2-hydroxybutanoate [4].

An interesting stereochemical feature of 2'-O-p-coumaroyl aloesins can be inferred by a comparative analysis of their proton n.m.r. data. Thus, the resonance frequencies of the p-coumaroyl group appear to occur in aloesin A and in iso-aloesin A at significantly higher fields than they do in the corresponding methyl p-coumarates. The aromatic protons in the ortho-positions to the side-chain of the cis-coumaroyl residue (H-5" and H-9") appear to be the most affected by the upfield shift. Taking into account that 8-C-glucosylflavones exist in two main rotational conformers [12], the plane of the pyranosyl ring being approximately perpendicular to the chromone nucleus, it is reasonable to assume that the same conformations occur in aloesins (Fig. 6). An inspection of molecular models reveals that the planes of the aromatic rings of the chromone system and of the p-coumaric residue can take a parallel orientation. The 2'-O-substituent is situated above or below the plane of the chromone nucleus, thus undergoing a shielding effect. Such an effect was reported for the signal of 2"'-O-acetyl group of various acetylated 8-C-glucosylflavones [13].

6-phenyl-2-pyrone

Aloenin A was isolated by Japanese researchers in 1978 from A. arborescens and found to have a marked inhibitory effect on the rat gastric secretion [14]. After a first structural hypothesis, which was then proved to be wrong, the correct formula reported in Fig. 7 was unequivocally demonstrated by X-ray crystallographic analysis [14] and by total synthesis of the aglycone [15].

Recently, as a part of our systematic chemical studies on aloe drugs of different origin, we were able to isolate a new water-soluble constituent of a commercial sample from Kenya. The structure of this product, we named it aloenin B, was established by the usual spectral methods and by chemical correlation with aloenin A [6].

A major difficulty in structural elucidation was the location of the p-coumaroyl group, for which the position adjacent to the anomeric center of both the β-D-glucosyl residues appeared a likely candidate on the basis of n.m.r. chemical shifts. Convincing evidence in favor of the structure indicated in Fig. 7, in which the non-acylated glucosyl group is linked to the 10-O-position, came from differential proton n.O.e. experiments performed on the deglucosyl-O-methyl aloenin B, as shown in Fig. 8. The intensity enhancement of both the singlets at δ 6.47 and 6.61, due to the aromatic protons in the 11- and 9-positions, respectively, allowed
Fig. 8 - Chemical transformations of aloecin B.
the irradiated methoxy group and, as a consequence, the non-acylated glucosyl residue of aloein B, to be located in the 10-position.

It is worth noting that a marked upfield shift appeared in the proton n.m.r. spectrum for the resonances of the pyrone ring protons (especially H-3), going from aloein B to its deacyl derivative, which was obtained by reaction with diazomethane in methanol. This fact can be interpreted in terms of a preferred conformation of the aloein molecule having the pyrone protons deshielded by the electron current of the benzene nucleus of the p-coumaroyl moiety (Fig. 9).

![Fig. 9 - Conformation of aloein B.](image)

**Anthracene derivatives**

Aloins A and B are the two diastereoisomers shown in Fig. 10. The absolute configuration of C-10 in each compound has been recently determined by us on the basis of a conformational study carried out using the n.O.e. technique [16]. The steric formula in Fig. 10 represents aloin A if R1 is H and R2 the hydroxymethyl group, and aloin B by inversion of the substituents.

The presence of the two aloins in Cape aloe was proved in 1979, when the drug was examined by high pressure liquid chromatography [17]. Previously, it was commonly thought there was just one aloin, known as barbaloin, which could be obtained from aloe by means of various treatments (precipitation as calcium salt, elimination of calcium with sulphuric acid, and so on), and final crystallization from methanol [18]. Its structure was clarified by Hay in 1956, apart from the configuration of the asymmetric carbon atom of the anthrone nucleus.

At present we know that crystalline barbaloin, on which all structural researchers have been carried out, is aloin A [19], that is the less polar diastereoisomer and the more largely retained in column in reverse phase liquid chromatography (Fig. 11).

The two aloins are easily interconvertible in weakly basic medium or in dimethylsulphoxide. The equilibrium mixture contains the two isomers in almost a 1:1 ratio, as occurs in aloe drug.
Biosynthetic studies on the origin of aloins in *A. arborescens* seem to indicate that aloin B is the true natural product, whereas its isomer is an artefact formed by a non-enzymatic equilibration during the processing of the plant material [20].

The most peculiar characteristic of the aloin molecule is the presence of a glucosidic group linked to the aglycone by means of a bond between two tetrahedral carbon atoms. It is the only example of an alkyl C-glycoside so far found in nature.

The two 11-O-rhamnosides of aloins, that is, aloinosides A and B, have also been found in a limited number of Aloe sp. [21]. The fifth 10-O-glucosylated anthrone, homonataloin, was first isolated from Natal aloe, a kind of drug no longer commercially used. This compound differs from the other anthrones in having a methoxy group at C-8 and an additional hydroxy group in 7-position [22].

Free anthraquinones seem to be present at low levels in aloes (Fig. 12). Aloe-emodin and, to a lesser extent, chrysophanol are typical and widespread in the genus, while the other 1-methyl- and 3-methylanthraquinones have been reported to occur only in *A. saponaria* [23].

Partially hydrogenated anthracene derivatives have been isolated from *A. sapo-
naria [24]. They are reported in Fig. 13. Their structural and biogenetic relationships with the anthraquinones of Fig. 12 are evident.

Four dimeric anthraquinone-anthrone and anthraquinone-anthraquinone have been described as metabolites of Aloe plants (Fig. 14) [25]. Their origin can be accounted for by assuming an intermolecular oxidative coupling between two anthracene units via an enzymatic phenol oxidation.

**Biogenesis**

From an inspection of the molecular structures of the natural products mentioned before, their polyketide origin is apparent.

---

**Fig. 11** - Liquid chromatogram of Cape aloe.
Every carbon skeleton can be derived from a proper assembly of two-carbon units followed by a particular cyclization [26].

Thus, all compounds can be grouped into three biogenetic families (Fig. 15):

i) the octaketide family, arising from 8 aceto-malonate units, comprises the two anthracene subfamilies (1-methyl and 3-methyl);

ii) the eptaketide family, arising from 7 aceto-malonate units, includes the largely populated subfamily of 5-methylchromones as well as that of naphtalene derivatives (represented by isoeleutherochromes only);

iii) the hexaketide family, arising from a precursor of 6 aceto-malonate units, is represented by 6-phenyl-2-pyrones.
Fig. 13 - Metabolites of A. saponaria.

Fig. 14 - Dimeric anthracene derivatives.
The variety of structures can be easily explained in terms of different folding of the poly-β-ketomethylene chain in the enzymic matrix [26].

This rationalization of the origin of typical Aloe metabolites appears greatly convincing. It indicates that the polyketide pathway can be regarded as a characteristic feature of Aloe sp., a feature of potential chemotaxonomic value.

However, in spite of this reasonable hypothesis, the biosynthetic scheme shown in Fig. 15 requires sound experimental proofs. It must be pointed out, in this regard, that precursor-incorporation experiments are still lacking.
REFERENCES

[16] Manitto P. et al., results to be published.